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Growth Performance, Caeca Microbial Population and Immune Response of Starter Broiler Chicks Fed Aqueous Extract of Balanites Aegyptiaca and Alchornea Cordifolia Stem Bark Mixture

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Research Article

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Abstract

A total of Two hundred and fifty (250), 1-day old (Cobb) broiler chicks with mixed sex were used to evaluate the the growth performance, caeca microbial population and immune response of starter broiler chicks fed aqueous extract of Balanites aegyptiaca and Alchornea cordifolia stem bark mixture (BACM). Birds were reared on a deep litter system and randomly divided into five treatment with five replicates consisting of 10 birds each in a completely randomized design. Treatment 1 (T1) were given basal diet + 0 % BACM, T2, T3, T4 and T5 were fed 20, 40, 60 and 80 ml/liter BACM respectively. The experiment lasted for 28 during which clean feed and water were offered ad libitum. The results obtained revealed that the average weight gain (AWG), feed conversion ratio (FCR) and mortality were influenced by the dietary treatments ($P<0.05$). Birds in T5 had the highest AWG and FCR (1159.3 g, 1.57) followed by T4 (1070.2 g, 1.70), T3 (1047.4 g, 1.74), T2 (981.1 g, 1.86) and T1 (850.7 g, 2.14) respectively. Activities of superoxide dismutase (SDA), glutathione peroxidase (GPx), catalase (CAT), malonyldialdehyde (MLA) and antibody titres against Newcastle and gumboro disease were significantly affected by BACM ($P<0.05$). Caeca microbial population of Escherichia coli and Lactobacilli were significantly different among the treatments ($P<0.05$). E. coli count in T1 were higher compared to other treatments ($P<0.05$), Lactobacilli population increased in T2, T3, T4 and T5 compared to T1. It was concluded that BACM can be fed to broiler chicks at 80 ml/litre without any negetive effect on the performance and immune response of birds.

Keywords: Broiler Chicks; Immune Response; Performance; Antibodies

Introduction

The use of medicinal plants of high therapeutic value have recently gained interest since the ban on the use of antibiotics by the European Union in 2006 due to anticipated toxicity, high cost and adverse effect [1]. According to Mahima, et al. [2], there are over 200,000 species of medicinal plant species where about 800 plant species have been used by different communities for curing different diseases and they contain several minerals, vitamins, protein and essential fatty acids. Among the potential medicinal plants are Balanites aegyptiaca and Alchornea cordifolia, they contains several bioactive chemicals or phytochemicals which allows them to perform multiple biological activities. The plants are found to contain alkaloids, flavonoids, phenols, saponins, tannins, oxalate, terpenoids, steroids etc. Alagbe, et al. [3] which confers them ability to function as an antimicrobial, anti-inflammatory, antiviral, antioxidant, anti-

ulcer, antihelminthic and anti-implantation [4-8].

Balanites aegyptiaca belongs to the family Zygophyllaceae. It's found in many parts of Africa (Kenya, Sudan, Somalia, Djibouti, Nigeria and Ethiopia) and Asia (China, India, Pakistan, Afghanistan, Sri Lanka and Bangladesh) [9]. The leaves are characterized by dark green or grey green colouration with two firm coriaceous leaflets spirally arranged on the shoots [8]. The leaf, stem bark and roots are traditionally used to treat headache, stomach disorder, wound; skin infection and tooth ache [10]. Phytochemical evaluation of the stem bark, leaves and roots revealed the presence of appreciable quantity of flavonoids, alkaloids, saponins, phenols, terpenoids, tannins glycosides, amino acids, vitamins, carbohydrates and protein [10].

Alchornea cordifolia (Euphorbiaceae) is a perennial evergreen tree which measures between 4-8 m high, grows near river bank or marshy places and it is characterized by unisexual and sessile flowers and wide spread in Kenya, Senegal, Nigeria, Niger, Cameroon, Tanzania, Angola, Mudagaska, China and some parts of India. Mamadou, et al. reported that A. cordifolia is loaded with several secondary metabolites (terpenoids, alkaloids, phenols, steroids, glycosides, hydroxybenzoic acid, tannins, imidazopyrimidine, alchorneine, namelygallic acid, anthralinic acid, ellagic acid, alchornidine guanidine, nutrients (carbohydrate, protein and amino acids) and have traditionally used to treat rheumatism, arthritis, tooth ache and pile.

Previous studies have shown that the immune system benefit greatly from proper nutrition (phytogenics) of the bird. Phytogenics is a safe growth promoter without side effects on birds, enhance the modulation of beneficial intestinal microbiota by controlling potential pathogens [11] and improvement of nutrient absorption and enzyme activity to enhance better weight gain and feed conversion efficiency due to the presence of several bioactive chemicals.

In view of these potential a synergistic combination of different plants will give better result, especially those that are underexplored. Therefore the experiment was carried out to evaluate the growth performance, caeca microbial population and immune response of starter broiler chicks fed aqueous extract of Balanites aegyptiaca and Alchornea cordifolia stem bark mixture.

Methodology

Experimental Site

The experiment was carried at Kano State University of Science and Technology, Wudil, Kano State, Nigeria.

Collection, Processing, Preparation of Extract and Analysis

Healthy stems of Balanites aegyptiaca and Alchornea cordifolia were obtained from the Teaching and Research farm of Kano State University, Nigeria. The plant materials were identified and authenticated by a botanist (Dr. Bashir), and thoroughly washed with distilled water to remove soil and other bound particles, air dried separately until a constant weight was obtained and made into powder using a pulverizer. Samples were later stored in a well labeled air tight container and kept for further analysis. 100 g of each ground sample (Balanites aegyptiaca and Alchornea cordifolia) were mixed together (1:1) dissolved in 1000 ml water, stirred continuously and kept in the refrigerator for 48 hours. The extract was filtered using Whatman filter paper No. 1 to obtain filtrate (BACM).

Proximate compositions of test material and experiment diet were determined by using official method of analysis by AOAC [12].

Phytochemical evaluation of tannins, alkaloids, saponins, flavonoids, phenols, oxalate, glycosides, steroids and terpenoids were estimated using methods described by Atamgba, et al. [13], Harbone, et al. [14], Shabbir, et al. [15], Odebiyi, et al. [16], Boham, et al. [17]. Mineral analyses were carried out using Atomic Absorption Spectrophotometer (AAS) model 12-0TA.

Animals and Their Management

A total of two hundred and fifty one-day old broiler chicks (Cobb) strain of mixed sex were randomly distributed into five treatments with 5 replicates, each replicates contained 10 birds each in a completely randomized design. Prior to the arrival of the birds the deep litter pen house were properly disinfected and the foot bath is constructed to ensure biosecurity. Birds were weighed on arrival to the farm to determine their initial body weight and weekly thereafter. Wood shavings were used as litter material and lighting was continuous, vaccines were administered according to the prevailing disease condition in the environment and all necessary management practices were strictly adhered to, clean feed and water were offered *ad libitum* and the experiment lasted for 28 days.

Feed Formulation and Experimental Set Up

A standard starter's ration was formulated to meet the nutritional recommendation of birds by NRC [18]. It was made up of corn-soya meal based diet and it contained 23 % crude protein and 2900 Kcal/kg energy.

- Treatment 1- Basal diet + 0 % BACM

- Treatment 2 – Basal diet + 20 ml/liter BACM
- Treatment 3 – Basal diet + 40 ml/liter BACM
- Treatment 4 – Basal diet + 60 ml/liter BACM
- Treatment 5 – Basal diet + 80 ml/liter BACM

Experimental Measurements

Performance Record

Feed intake was recorded daily and body weight gain was recorded weekly, feed conversion ratio was calculated by dividing the total feed intake by weight gain, mortality was also recorded as it occurs.

Fatty Acid of the Feed

Fatty acid composition of the feed was carried out using gas liquid chromatography (Model 231 A-01, Punjab, India). Percentage concentrations were evaluated according to the methods outlined by Suriya, et al. [19].

Haemagglutination Inhibition Test

Birds were orally vaccinated against Newcastle on the 5th and 18th day and Gumbroro diseases on the 11th and 23rd day. Three birds were randomly selected per replicate to access the antibody response to Newcastle and Gumboro virus on the 20th and 28th days of the experiment. Analysis was done according to the method described by Thayer, et al.

Caecal Microbial Population

At the end of the experiment (12 weeks), caeca microbial count was conducted using five (5) grasscutters per treatments, caeca contents were collected from slaughtered animal and 10-fold serial dilution method, in which of 1% peptone solution was mixed with caeca samples and poured on Mac Conkey agar plates and lactobacilli medium III agar plates, was used to determine the colony forming unit (cfu) in each gram of caeca sample by means of pour plate method. Colonies of *E. coli* and Lactobacilli were enumerated according to the method outlined by Phyo, et al. The microbial counts were determined as colony forming units (Cfu/g) of sample.

Antioxidant Status

Activity of superoxide dismutase (SDA), glutathione peroxidase (GPx), catalase (CAT) and malonyldialdehyde (MLA) were carried out using method outlined by Mahipal, et al.

Statistical Analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan multiple range tests [20]. Significant was declared if P ≤ 0.05 (Tables 1-7).

Ingredients	Quantity (kg)
Maize	52
Wheat offal	5.24
Soya meal	38
Fish meal (72%)	3
Bone meal	0.5
Limestone	0.25
Lysine	0.2
Methionine	0.25
Premix	0.25
Salt	0.3
Toxin binder	0.01
Total	100
Analyzed nutrient (%)	
Crude protein	23.11
Crude fibre	3.09
Ether extract	5.12
Calcium	0.97
Phosphorus	0.46
Energy (kcal/kg)	2990.7

Table 1: Composition of experimental diet.

Fatty acids	% composition
TSFA	51.44
TUFA	45.62
MUFA	38.06
PUFA n-3	1.01
PUFA n-6	10.22
n-3: n-6	10.11
Ant. Index	0.21

Table 2: Fatty acid composition of the experimental diet.

[1]Total saturated fatty acid= C12:0 + C14:0 + C16:0 + C18:0 + C20:0 +C22:0

[2]Mono unsaturated fatty acid= C14:1$_c$ + C16:1$_c$ + C18:1$_c$ + C18:1n9t + C18:1n9c + C22:1

[3]Polyunsaturated fatty acid = C18:2 n6 + C20:5 n3 + C18:3n3 + C20:4n6 + C20:3n6 + C: 22:6n3

[4]n-6: n-3 = (C18:2 n6 + C20:4n 6 + C20:3n 6 / (C20:5n3 + C18:3n 3 + C: 22 6n 3), [5]Antherogenic index = (C12:0+ 4×C14:0+ C16)/∑ of UFA.

Parameters (%)	*Balanites aegyptiaca* stem bark	*Alchornea cordifolia* stem bark	Permissible range (%)
Alkaloids	2.33	0.42	9.13
Hydrolysable tannins	3.07	2.44	4.56
Condensed tannins	0.01	0.03	1.88
Flavonoids	5.84	6.1	12.1
Saponins	0.36	0.22	7.02
Phenols	2.88	1.84	-
Terpenoids	0.6	0.93	-
Steroids	1	0.04	-
Phytates	0.07	0.15	2.13
Oxalates	0.05	0.02	0.54

Table 3: Phytochemical composition of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark.

Parameters	T1	T2	T3	T4	T5	SEM
IBW (g)	41.65	41.03	41.29	41.4	41	0.02
FBW (g)	892.3^{c}	1022.1^{b}	1088.7^{b}	1111.6^{a}	1200.3^{a}	0.19
WG (g)	850.7^{c}	981.07^{b}	1047.4^{b}	1070.2^{a}	1159.3^{a}	0.12
ADWG (g)	30.38^{c}	35.04^{b}	37.40^{b}	38.22^{a}	41.40^{a}	0.03
FI (g)	1822.1	1820.9	1820.5	1820.1	1820	0.25
ADFI (g)	65.08	65.03	65.02	65.01	65	0.06
FCR	2.14^{a}	1.86^{b}	1.74^{b}	1.70^{b}	1.57^{c}	0.52
MORT.	3	-	-	-	-	-

Table 4: Performance characteristics of broiler chicks fed different levels of BACM.
Means in the same row with different superscripts differ significantly ($P<0.05$)
IBW: Initial body weight; FBW: final body weight; WG: weight gain; ADWG: average daily weight gain; FI: feed intake; ADFI: average daily feed intake; FCR: feed conversion ratio; MORT: mortality.

Parameters Cfu/g	T1	T2	T3	T4	T5	SEM
E. coli	17.80^{a}	12.04^{b}	10.28^{b}	9.14^{c}	9.01^{c}	0.45
Lactobacilli	20.01^{c}	29.18^{b}	30.16^{a}	33.45^{a}	35.02^{a}	1.57

Table 5: Caeca microbial population of broiler chicks fed different levels of BACM.
Means in the same row with different superscripts differ significantly ($P<0.05$)
SEM: Standard error of mean

Parameters	T1	T2	T3	T4	T5	SEM
MLA (U/mg Hb)	1.03^{c}	2.11^{b}	2.16^{b}	3.00^{a}	3.03^{a}	0.01
SDA (U/mg Hb)	21.5^{b}	24.1^{b}	30.0^{a}	33.8^{a}	35.0^{a}	2.04
GPx (U/mg Hb)	14.5^{b}	16.1^{b}	23.6^{a}	24.0^{a}	28.1^{a}	1.51
CAT (U/mg Hb)	41.0^{a}	38.1^{b}	30.8^{b}	30.0^{b}	29.5^{b}	1.94

Table 6: Antioxidant status of broiler chicks fed different levels of BACM.
Means in the same row with different superscripts differ significantly ($P<0.05$)
SOD, superoxide dismutase; CAT, catalase; MLA, malondialdehyde; GSH, reduced glutathione
SEM: Standard error of mean

Parameters	Day	T1	T2	T3	T4	T5	SEM
Newcastle (Log_2)	5	2.44^c	3.67^b	4.06^a	4.22^a	4.33^a	0.05
	18	3.03^c	4.03^c	6.07^b	6.45^b	7.11^a	0.28
Gumboro (Log2)	11	1.78^b	2.01^a	2.67^a	2.89^a	3.00^a	0.03
	23	2.56^c	3.96^b	4.22^b	5.51^a	5.05^a	0.37

Table 7: Antibody titres of broiler chicks fed different levels of BACM.
Means in the same row with different superscripts differ significantly ($P<0.05$)
SEM: Standard error of mean

Results

Proximate and Fatty Acid Composition of Experimental Diet

The chemical composition of experimental diet is presented in Table 1. Result revealed the presence of crude protein (23.11 %), crude fibre (3.07 %), ether extract (5.12 %), calcium (0.97 %), phosphorus (0.46 %) and energy (2990.7 Kcal/kg). Similarly, total saturated fatty (TSFA) 51.44 %, total unsaturated fatty acid (TUFA) 45.62 %, monosaturated fatty acid (MUFA) 38.06 %, omega 3 fatty acid (n-3) 1.01 %, omega 6 fatty acid (n-6) 10.22 %, n-3 : n-6 (10.11 %) and antheriogenic index (0.21 %) is presented in Table 2.

Phytochemical Analysis of Balanites Aegyptiaca and Alchornea Cordifolia Stem Bark

Phytochemical composition of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark is presented in Table 3. *Balanites aegyptiaca* stem bark contained alkaloids (2.33 %), hydrolysable tannins (3.07 %), condensed tannins (0.01 %), flavonoids (5.84 %), saponins (0.36 %), phenols (2.88 %), terpenoids (0.60 %), steroids (1.00 %), phytates (0.07 %) and oxalates (0.05 %) while *Alchornea cordifolia* stem bark posses alkaloids, flavonoids, saponins, condensed tannins, hydrolysable tannins, phenols, steroids, oxalates and phytates at 0.42 %, 6.10 %, 0.22 %, 0.03 %, 2.44 %, 1.84 %, 0.04 %, 0.02 % and 0.15 % respectively.

Performance Characteristics of Birds Fed Different Level of BACM

Performance characteristics of the experimental birds are presented in Table 4. IBW values ranged between (41.00 – 41.65 g), FBW (892.3 – 1200.3 g) and WG (850.7 – 1159.3 g) were higher ($P<0.05$) in T5 than in T4, T3, T2 and T1. FI values ranged between (1820 – 1822 g) and ADFI (65.0 – 65.08 g), however, no significant differences were observed among the treatments ($P>0.05$). Mortality were highest for T1 and none was recorded in the other treatments ($P<0.05$).

Caeca Microbial Population of Broiler Chicks given BACM

Caeca microbial population of birds fed BACM is presented in Table 5. *E. coli* values ranged between (9.01 – 17.80 Cfu/g) and lactobacilli (20.01 – 35.02 Cfu/g). *E. coli* were lowest in T5, T4, T3 and T2 and highest in T1 ($P<0.05$). Lactobacilli count were highest in T4 and T5 and lowest in T1 ($P<0.05$).

Immune and antioxidant status of broiler chicks fed BACM

The antioxidant status of the experimental birds is presented in Table 6. Whereas MLA (1.03 – 3.03 U/mgHb), SDA (21.5 – 35.0 U/mgHb) and GPx (14.5 – 28.1 U/mgHb) were lowest ($P<0.05$) for T1. CAT (29.5 – 41.0 U/mgHb) were highest ($P<0.05$) for T1 relative to other treatments.

The antibody titre as influenced by BACM is presented in Table 7. Newcastle antibody titre in day 5 ranges between 2.44 – 4.33 (Log_2) while day 18 3.03 – 7.11(Log_2). Parameters were significantly different among the treatments ($P<0.05$). Gumboro antibody titers on day 11 ranges between 1.78 – 3.00 (Log_2) while on day 23 (2.56 – 5.05) (Log_2) were affected by feeding BACM to birds ($P<0.05$).

Discussion

The chemical composition of experimental diet is in agreement with the nutritional requirement of birds according to NRC [18]; Aduku, et al. [21]. This is an indication that the feed contains the entire nutrients necessary for optimum growth and immunocompetency of animals [22,23]. Phytochemical composition of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark reveals the presence of several bioactive chemicals or secondary metabolites which performs multiple biological activities. The present findings coincides with other research findings from Ngaha, et al. [5,24,25]. The presence of alkaloids confers the stem bark ability to function as an antibacterial, anti-malarial and anticancer; this supports the earlier findings of Eldin,

et al. Flavonoids play a pivotal role as an anti-inflammatory, anti-allergic and anti-plasmodic [9,10]. Saponin performs both antibacterial and antifungal activities Alagbe, et al. 26. Phenols are strong antioxidants which prevents the entry of diseases [27]. Terpenoids has high therapeutic value and function as antimicrobial, anticarcinogenic and anti-diuretic. Steroids play a major role in fertility of animals [13,26]. Tannins have found therapeutic application as antiviral and antibacterial [28]. Phytate are antioxidant compounds capable of binding minerals. Bioactive chemicals in plants vary according to species, age, soil type, geographical area and method of extraction [29]. Olanipekun, et al. [30]reported a higher value of 6.78 % (alkaloids) and 2.79 % (saponins) in Morinda lucida stem bark. Enin, et al. [31] also reported a lower value of 0.60 % (tannins), 0.52 % (alkaloids), 0.31 % (flavonoids), 0.65 % (saponins), 0.14 % (oxalate) and 0.21 % (phytate) in Sida acuta. However, all the values obtained in this study were within the tolerable level reported by Olafadehan, et al. [32], Alagbe, et al. [33].

The fatty acid in the diet shows that it's loaded with poly unsaturated fatty acid; this removes the risk of cardiovascular infection and ensures food safety (meat). Feeding animals with the experimental diet and BACM will improve the nutritive value of the meat, since phytochemicals in plants can function as modulators, this result is consistent with the reports of Suriya, et al. [19]; Alagbe, et al. [34]. Low antheriogenic index reduces the risk of artheriosclerosis [35]. Omega -3 and omega - 6 polyunsaturated fatty acid ratios was within the range recommended by Simopoulos, et al. [36].

The improved the final live weight, total weight gain and average daily gain of birds in treatment 4 and 5 compared to the other treatments could be attributed to efficient feed utilization as a result of various phytochemicals in BACM, these bioactive chemicals also enhanced feed conversion ratio (FCR) in the group. The result obtained is in accordance with the reports of Fascina, et al. [37]; when phytogenic additives were fed to broiler chickens. Similar observation was recorded by Dingfa, et al. [38] when turmeric rhizome extract was supplemented of Wenchang broiler chickens. According to Krishnan, et al. intestinal microorganisms play a key role in nutrient absorption and modulating the immune system and metabolic signaling pathways. Hyun, et al. [39]; Michiels, et al. [40]; Shittu, et al. [41] reported that phytogenic feed additives can reduce the activity of pathogenic bacteria by competitive exclusion due to the presence of phenols, alternation of bacteria cells and preventing the development of virulence structures in pathogenic microorganisms. This could be one of the reasons why mortality was not recorded in treatments fed BACM, the test material have also proven its ability to repopulate the beneficial bacteria (Lactobacilli) to maintain dysbiosis. This result is in consonance with the findings of Han, et al. on the influence of antimicrobial feed additives on broiler commensal post hatch gut microbiota development.

According to Peréz, et al. [42] oxidation occurs during the transfer of electrons from one atom to the other essential for cell metabolism with oxygen as an electron acceptor releasing energy in the form of Adenosine triphosphate. Free radicals are generated during a break in the process predisposing animals to stress and disease but are naturally scavenged from cells by serum antioxidant enzymes i.e., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [40,43]. Phytogenics have been reported to contain antioxidants (phenol and flavonoids) giving total protection to the body and its metabolism against free radicals in the body, thus improving the health status of birds [3,32]. These phytochemicals are also responsible for the rise in serum antibody titres and hormonal immunity especially among birds in T4 and T5. This result in this study is in accordance with the work of Fuluyi, et al. [44]; Olugbemi, et al. [45].

Conclusion

The use of phytogenic feed additives are one of the ways to ensure food safety, maximize potential and put an end to the leading worrying increase in cases of antibiotic resistance diagnosed in animals and humans through direct contact, environmental contamination and feed consumption [46]. Medicinal plants are of high therapeutic value, relatively cheap, safe and effective. It was concluded from this experiment that BACM can be fed to broiler chicks at 80 ml/liter of water without any deleterious effect on the performance and health status of animals.

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